



# Simple Scale-up on a 940-LC Analytical to Preparative HPLC

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## Introduction

Preparative chromatography is the process of collecting the eluent from an HPLC. The quantity of sample collected may vary from micrograms to kilograms; however, the aim at any scale is to attain a more purified material rather than analyzing a sample's composition.

Once an analytical separation has been developed for a sample, modifications are required to the instrumentation and method. Parameters such as flow rate, column size and sample injection volume are changed using a linear scale-up factor to produce an effective preparative HPLC separation.

A linear scale-up factor is the ratio of the cross sectional areas of the analytical column and the intended preparative column.

$$\text{Factor} = r^2(\text{prep})/r^2(\text{analytical})$$

Where:  $r$  is the radius of the column.

When scaling up from a 4.6 mm ID analytical column to a 21.2 mm ID preparative column, the linear scale-up factor would be:

$$(21.2\text{mm}/2)^2/(4.6\text{mm}/2)^2=21.24$$

In the simplest case, this factor is applied to flow rate and sample injection volume.

Other parameters such as column length, packing material, sample concentration and run time are held constant.

In general, to perform the scale-up process:

1. Optimize the preparative separation on an analytical column. Check sample solubility in mobile phase.
2. Multiply all of the scaleable elements of the system by the appropriate linear scale-up factor. Do not change any of the 'absolute' elements.
3. Perform the preparative separation.

Where yields greater than that achievable with linear scale up process are required, a common practice with preparative HPLC is to overload the column. This is achieved in 2 ways:

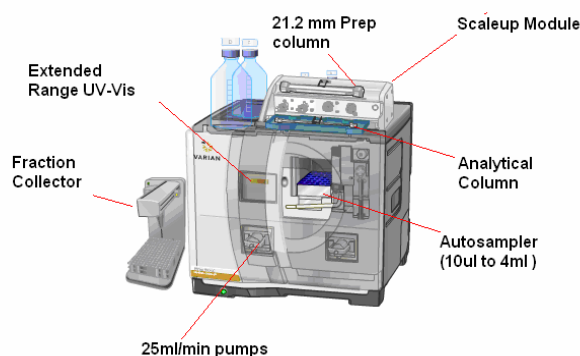
- Increasing the concentration of the material injected (where solubility permits)
- Increasing the injection volume

Column overloading can result in severely distorted peak shapes. To effect satisfactory collection requires accurate and

reproducible real-time peak detection algorithms for use with the fraction collector.

This application note will show how the Varian 940-LC Analytical to Preparative Liquid Chromatograph can quickly and easily scale-up from analytical to preparative scale.

## Instrumentation



The Varian 940-LC Analytical to Preparative Liquid Chromatograph, Varian 445-LC Scale-Up Module and Varian 440-LC Fraction Collector are designed to simplify and automate the analytical to preparative LC scale-up process.

The Varian 445-LC Scale-Up Module is a fully automated unit allowing switching between analytical and preparative columns. This software-controlled module switches from analytical to preparative operation. Optimization of the flow path from analytical to preparative ensures that, when moving from one to the other, the analytical and preparative chromatograms are correlated.

## Materials and Reagents

Mobile Phases:

HPLC Grade LiChrosolv Acetonitrile was purchased from Merck, Kilsyth, Victoria, Australia and deionized water (18 MΩcm Milli-Q), Millipore.

Chemicals:

Uracil, acetophenone, methyl benzoate, toluene and naphthalene were purchased from Sigma-Aldrich Inc.

## Sample Preparation

Table 1. Sample composition

Sample 1 composition for analytical injection	Uracil 40 mg/L Acetophenone 60 mg/L Methyl benzoate 60 mg/L Toluene 3g/L Naphthalene 500 mg/L
Sample 2 composition for preparative injection	10 x sample 1
Solvent	HPLC grade acetonitrile

## Chromatographic Conditions

The conditions for the analysis are summarized in Table 2.

Table 2. Chromatographic conditions

	Analytical conditions	Preparative conditions
Column	Pursuit™ XRs C18, 5 µm, 4.6 mm ID x 150 mm length	Pursuit XRs C18, 5 µm, 21.2 mm ID x 150 mm length
Flow rate	1.0 mL/min	21.2 mL/min
Sample injection volume	20 µL	420 µL
Mobile phase	60:40 Acetonitrile/H <sub>2</sub> O	60:40 Acetonitrile/H <sub>2</sub> O
Collection mode	N/A	Threshold
Trigger parameter	N/A	40 mAu
Tube size	N/A	50 mL

## Results and Discussion

A 20 µL injection of a 5 component organic chemical mix (sample 1) was made and run at 1 mL/min on a 4.6x150 mm Pursuit XRs C18 column 5 µm attached to the analytical channel of a Varian 940-LC Analytical to Preparative Liquid Chromatograph.

The Varian 940-LC was then switched to preparative mode and a 420 µL (the analytical injection volume, 20 µL, multiplied by the scaling factor 21) injection was made at 21 mL/min on a 21.2x150 mm Pursuit XRs C18 5 µm column attached to the preparative channel of the Varian 940-LC.

The comparison of the two runs can be seen in Figure 1.

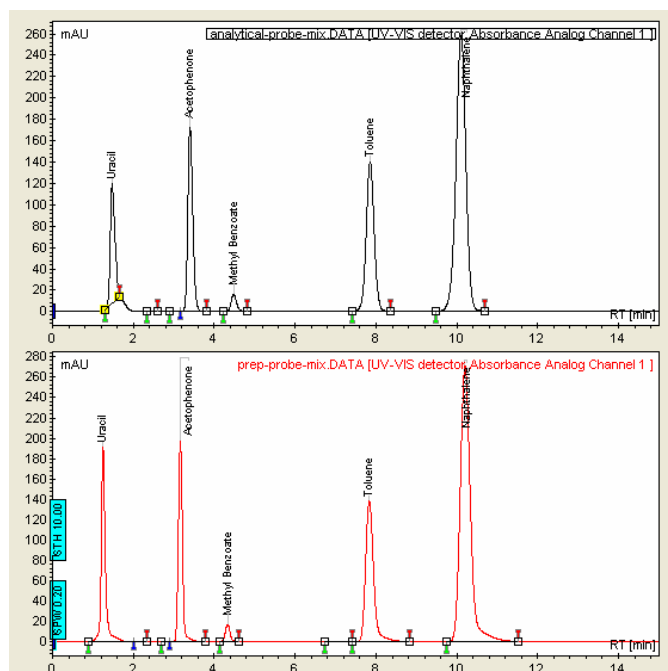


Figure 1. Upper chromatogram: Pursuit XRs C18 5 µm 4.6 mm x 150 mm, 1 mL/min and 20 µL injection volume. Lower Chromatogram: Pursuit XRs C18 5 µm 21.2 mm x 150 mm, 21 mL/min and 420 µL injection volume

Peak retention times, peak height (mAU) and overall chromatogram shape are well correlated.

A 1 mL injection of sample 2 was made onto the preparative column and this run was fractionated. The results can be seen in Figure 2.

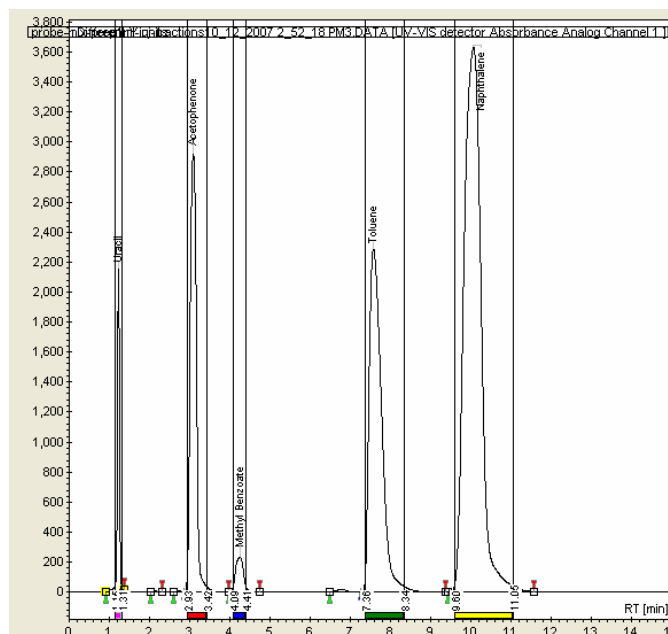


Figure 2. 1 mL injection of sample 2 onto a preparative column with a flow rate of 21 mL/min. Color-coded bars indicate the fraction and vertical lines indicate fractionation times.

Galaxie™ Chromatography Software has a collection log function which uses color coding to identify which fraction from the chromatogram is in which tube on the fraction

collector. Figure 3 shows the collection log for the preparative scale run.

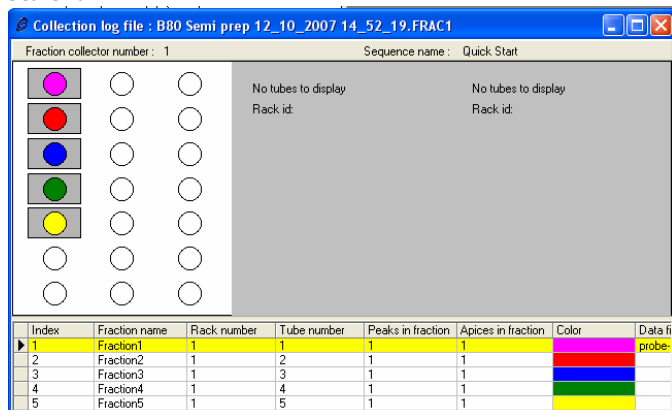


Figure 3. Fraction collection log

## Analysis of Collected Fractions

The resulting fractions from the 1 mL injection of sample 2 were then run on a Varian 920-LC Analytical Liquid Chromatograph with a PDA detector and compared to a spectral library derived from a chromatogram of the original sample to gauge peak purity. An example of a library search can be seen in Figure 4 showing an excellent match for naphthalene in fraction 5 indicating high peak purity.

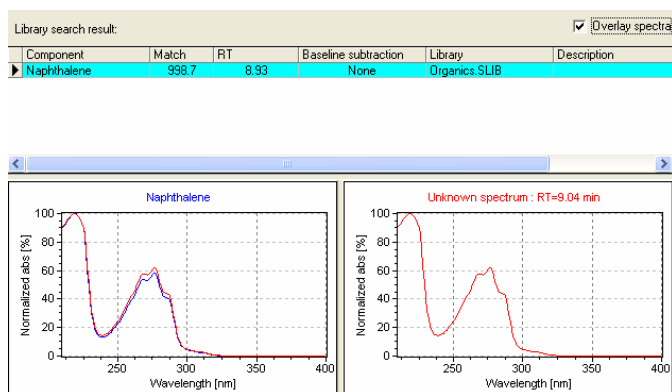


Figure 4. The results of a library search for a re-injection of fraction 5 with a 998.7 match for naphthalene

Another approach for determining peak purity uses all the spectra within a given peak to visualize, directly on the peak, pure, medium and impure regions.

See page 85 of the Galaxie PDA manual for details of the algorithms used. This approach is illustrated in Figure 5 confirming the peak homogeneity of fraction 5 (naphthalene).

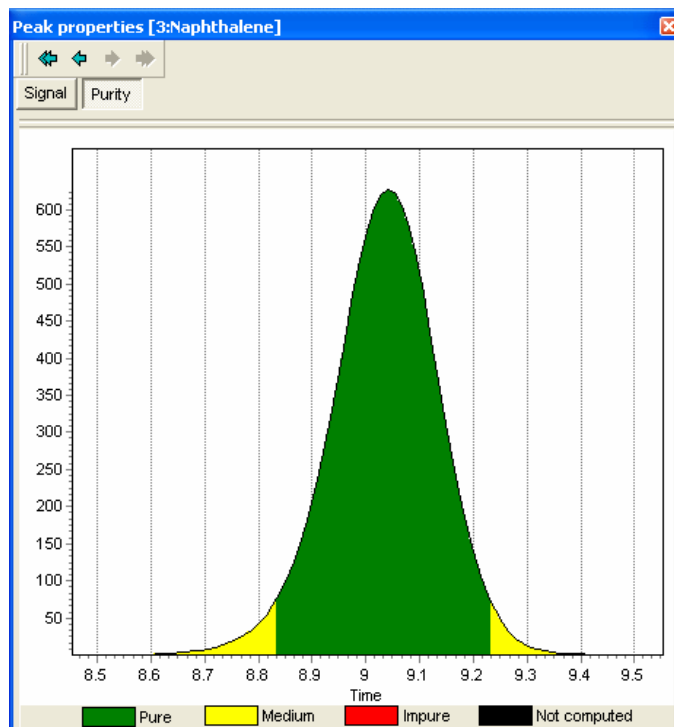


Figure 5. Peak purity map for fraction 5 – naphthalene

## Column Overloading with Large Volume Preparative Injection

To test the separation and fraction collecting capability of the Varian 940-LC when overloading a column, a 4 mL sample was injected into the preparative column on the Scale-Up Module and the resulting chromatogram is shown in Figure 6.

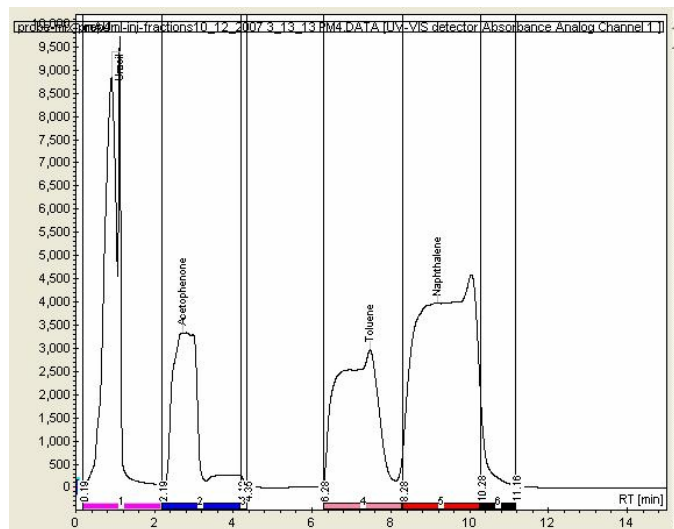


Figure 6. 4 mL injection of sample 2 onto 150x21 mm column at 21 mL/min

Owing to solubility issues sample 2, was dissolved 100% acetonitrile as a solvent instead of the mobile phase (60% ACN). This caused band broadening on the front of the column resulting in the broad peaks seen on the chromatograph in Figure 6. The 9.5 AU uracil peak at 1 minute shows the extended range capability of the Varian 940-LC dual pathlength flow cell.

The dual pathlength flow cell can handle up to a maximum 30 AU. Typically, high concentration samples increase absorbance to the point of saturation. The light path becomes opaque (%T goes to near zero) and the signal output is truncated at the maximum absorbance. Any signal beyond this point is lost until the absorbance reduces enough to allow transmittance.

The usual solution is to reduce sample concentration or change to a flow cell with a shorter light path. The flow cell with the short flow path will be less sensitive but its saturation point will be much higher. The ideal solution for very high absorbing samples would be a flow cell with 2 flow paths, providing dual-path operation, which would switch automatically to the short path when the concentration became too high on the long path. The dual pathlength flow cell used in this scale-up application, with a long pathlength of 4 mm and a short pathlength of 0.25 mm, provides the needed dual-path operation.

## Conclusion

The Varian 940-LC Analytical to Preparative Liquid Chromatograph with the Varian 445-LC Scale-Up Module and the Varian 440-LC Fraction Collector simplifies the development of preparative chromatography by switching seamlessly between analytical mode and preparative mode.

Careful optimization of plumbing in both the analytical and preparative flow paths ensures that a linear scale-up results in high levels of consistency between chromatography. This simplifies isolation and collection when operating in preparative modes.

The huge dynamic range of the dual path length flow cell design allows the user to see chromatographic details of highly absorbing samples usually obscured by detector saturation. Even in overloaded chromatographic conditions, the Varian 440-LC Fraction Collector is able to accurately determine and collect peaks throughout the entire run.

Using the PDA detector with the Varian 940-LC and Varian 445-LC Scale-Up Module enhances confidence in peak purity.

The results show that using the Varian 940-LC Analytical to Preparative Liquid Chromatograph with the Varian 445-LC Scale-Up Module and the Varian 440-LC Fraction Collector maintains both analytical and preparative scale performance integrity.

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*These data represent typical results.*

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